the $cTnT_{T204E}$-mediated effects on cardiac contractile function. Our novel observations have clinical relevance because increased activity of various PKC isoforms and upregulation of β-MHC are observed in failing human hearts.

3890-Pos Board B618

Force-Sarcomere Length Relations in Patients with Thin Filament Myopathy Caused by Mutations in NEB, ACTA1, TPM2 and TPM3

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Mutations in the nebulin gene (NEB), skeletal muscle alpha-actin1 gene (ACTA1), beta-tropomyosin 2 gene (TPM2) and alpha-tropomyosin 3 gene (TPM3) lead to thin filament myopathies, such as nemaline myopathy (NM), congenital fiber type disproportion (CFTD) and cap disease (CAP). A hallmark feature of these myopathies is muscle weakness. Here, we aimed to elucidate the effect of NEB, ACTA1, TPM2 and TPM3 mutations on thin filament length by determining the sarcomere length-dependence of force.

Quadriceps biopsies from NM, CFTD, and CAP patients (n=18) with mutations in the NEB, ACTA1, TPM2 or TPM3 were compared to biopsies from controls (n=3). Using permeabilized muscle fibers, maximal active tension was determined at incremental sarcomere lengths (range 2.0-3.5 μm) to obtain the force-sarcomere length relationship.

The maximal active tension (Fmax (in mN/mm2, mean ± SD)) was significantly lower in biopsies from severe NEB (18 ± 5), mild NEB (76 ± 5), severe ACTA1 (54 ± 13) and severe TPM3 (95 ± 14) patients compared to biopsies of controls (164 ± 17), whereas no significant changes in Fmax were observed in biopsies from mild ACTA1 (139 ± 27), mild TPM2 (1201 ± 8) and mild TPM3 (156 ± 13) patients. The classification of severity is based on the age of onset. No shift in the force-sarcomere length relationship was observed in mild ACTA1, TPM3 and TPM2 patients. Interestingly, in contrast to patients with ACTA1, TPM2 and TPM3 mutations, fiber preparations from both mildly and severly affected NEB patients showed a leftward shift of the force-sarcomere length relationship (a leftward shift of the force-sarcomere length relationship indicates shorter thin filaments).

Our data suggest that mutations in NEB result in the most pronounced changes in thin filament length. Insights in the mechanisms underlying weakness in patients with thin filament mutations are necessary to improve specific treatment strategies.

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Prolonged Relaxation Kinetics in Distal Arthrogryposis Skeletal Muscle Myofibrils with a MYH3 R672C Mutation

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The mechanisms underlying most forms of Distal Arthrogryposis (DA), a group of congenital contracture syndromes, are unknown. Our previous functional studies from adult individuals with DA caused by the heterozygous embryonic myosin heavy chain mutation, *MYH3* R672C, showed that the time required by DA skinned skeletal myofibers to relax completely after calcium-induced contraction was several-fold longer than controls (Racca *et al*. (2010) *Biophys J* 98:542-3a). Here we measured force and kinetics of activation & relaxation, and compared chemomechanical analysis using myofibrils and myofibers sampled from gastrocnemius muscle from two affected individuals vs. three control (non-DA) individuals. The prolonged relaxation was reflected in isolated myofibrils from DA patients, as 50% relaxation time from maximal activation was 36% longer, and 90% relaxation time was prolonged by 58%. The kinetics of the slow phase of relaxation were significantly slower, in both the rate and duration (DA: $k_{REL,SLOW}$=0.36 ± 0.07s^{-1};$t_{REL,SLOW}$=285 ± 21ms vs. Control: 0.79 ± 0.18s^{-1};199 ± 23ms), implying slower cross-bridge release. Use of ADP prolonged relaxation of control samples to a greater extent than DA preparations, suggesting that slower ADP release from myosin in DA myofibrils may be the mechanism of slower relaxation and cross-bridge release. We also found that both mRNA and protein for this "embryonic" myosin (gene *MYH3*) were present in adult skeletal muscle, such that a small amount of this slower myosin may prolong relaxation. Although the mechanism that leads to the congenital contractures must begin prenatally, these results suggest that *MYH3* R672H also affects ongoing function of adult skeletal muscle. Understanding the mechanism by which myosin mutations affect muscle cell contractility could provide a model for exploring the pathogenesis of more common contractures such as idiopathic clubfoot and facilitate the development of novel therapeutic approaches. **Supported by** F31AR06300(A.R.), 5K23HD057331(A.B.), HD048895(M.B.,M.R.).

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Functional Effects of the β-Myosin Mutation Arg453Cys in Familial Hypertrophic Cardiomyopathy

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In Familial Hypertrophic Cardiomyopathy (FHC) 1/3 of the patients are affected by mutations in the ß-myosin heavy chain (ß-MyHC), the myosin isoform of the ventricle and of slow skeletal muscle fibers in humans. Yet, not much is known about (i) direct effects of specific ß-MyHC-mutations on acto-myosin function, and (ii) how these mutations may trigger development of the FHC-phenotype.

To address these questions, we analyzed the effects of the ß-MyHC-mutation R453C on contractile properties of slow (type I) fibers from the *M. soleus* of a severely affected FHC patient. We found an about 12% higher isometric force, 15% faster rate constant of force redevelopment (k_{tr}), 10% higher isometric ATPase activity and essentially unchanged tension cost. Together with only slightly higher fiber stiffness in rigor, a main part of the increase in isometric force appears to be due to altered cross-bridge cycling kinetics, specifically an increase in f_{app}, the rate constant for the cross-bridge transition into force generating states. Currently we investigate whether increased force generated per myosin head e.g., by a larger y_0-value also contributes.

Relative quantification of mutated vs. wildtype ß-MyHC-mRNA of the heterozygous patient revealed a fraction of about 35% for the R453C-mRNA. Assuming similar abundance of mutated MyHC at the protein level, as we had found in other FHC-mutations, about a third of the ß-myosin heads in the sarcomeres carry the mutation. Thus, force contribution of the mutated myosin head population is about 40% increased. A larger variability in pCa_{50} among individual fibers with mutation R453C vs. control fibers, as we had seen for other FHC-mutations, suggests unequal expression of mutated myosin. We hypothesize that unequal expression of mutated myosin in individual cardiomyocytes causes imbalanced force generation and initiates functional impairment of the myocardium in FHC.

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The Structure-Function Analysis of Myosin Pseudo-Phosphorylation in Mouse Model of FHC

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Familial hypertrophic cardiomyopathy (FHC) is a disease of the heart caused by autosomal dominant mutations in genes coding for all major sarcomeric proteins, including the myosin regulatory light chain (RLC). In this report, we have explored the rescue strategies to ameliorate the malignant cardiomyopathy phenotype induced by an aspartic acid to valine substitution (D166V) in the RLC. Previous studies on porcine reconstituted preparations showed that the phosphorylation mimic (S15D) in the background of the D166V mutation (S15D-D166V) restored the calcium sensitivity of force and improved V_{max} of myosin ATPase that were largely compromised by the D166V mutation. Transgenic "Rescue Mice" carrying the S15D-D166V mutation have been generated and subjected to structural and functional measurements. Small angle X-ray studies on freshly skinned papillary muscle fibers revealed that a D166V-induced reorganization in cross-bridge mass distribution was partially reversed in Rescue Mice (abnormally increased $I_{1,1}/I_{1,0}$ ratio observed in Tg-D166V fibers returned to the value near Tg-WT). Noteworthy, pseudo-phosphorylation of D166V significantly restored fiber elasticity allowing for changes in the cross-bride mass distribution on stretch. *In vivo* cardiac morphology and function were assessed by non-invasive echocardiography followed by invasive left ventricular pressure-volume measurements (P-V loops). Echocardiography assessment confirmed hypertrophy in Tg-D166V mice showing an increased posterior wall thickness in systole. Invasive hemodynamics showed diastolic and systolic dysfunction in Tg-D166V mice. The end-systolic P-V relationship, a measure of heart contractility, which was largely reduced in Tg-D166V mice was completely ameliorated in Rescue Mice. In addition, the maximum dP/dt - End-Diastolic Volume relation, which was compromised in Tg-D166V was fully reversed in Rescue Mice. In conclusion, our results suggest that pseudo-phosphorylation of myosin RLC can mitigate both the structural and functional abnormalities of the FHC heart. Supported by NIH-HL108343 and HL071778 (DSC).